PB81-136517

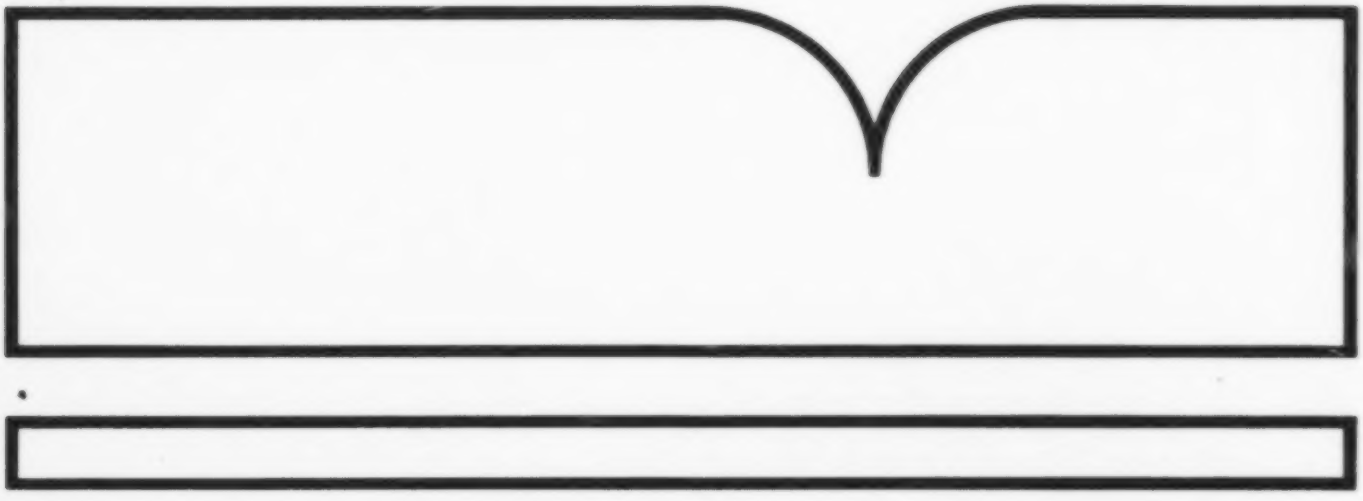
Modifications of the Tentative Standard Method for Improved Virus Recovery Efficiency

North Carolina Univ. at Chapel Hill

Prepared for

Health Effects Research Lab.
Cincinnati, OH

1980



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before comple

1. REPORT NO.	2.	3
EPA-600/J-80-195	Journal ARticle	PB81-136517

4. TITLE AND SUBTITLE	5. REPORT DATE
Modifications of the Tentative Standard Method for Improved Virus Recovery Efficiency	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) M.D. Sobsey, J.S. Glass, R.R. Jacobs, W.A. Rutala	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept. of Envir. Sci. and Engrg. School of Public Health Univ. of North Carolina Chapel Hill, N.C.	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO. R804218
12. SPONSORING AGENCY NAME AND ADDRESS USEPA Health Effects Research Laboratory Cincinnati, Ohio 45268	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

Published in: Journal American Water Works Assn., V.72(6):350-55 (June 1980)

16. ABSTRACT

Deficiencies in the tentative standard method (TSM) for concentrating viruses from water (explored by the authors in the May Journal) can be overcome or circumvented by certain procedural modifications. These modified procedures can improve virus adsorption by polyvalent cation enhancement or the use of alternative adsorbent filters, and can also enhance elution of adsorbed viruses.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

EPA-600/J-80-195
Journal Article

Modifications of the Tentative Standard Method for Improved Virus Recovery Efficiency

Mark D. Sobsey, J. Steven Glass, Robert R. Jacobs, and William A. Rutala

Deficiencies in the tentative standard method (TSM) for concentrating viruses from water (explored by the authors in the May JOURNAL) can be overcome or circumvented by certain procedural modifications. These modified procedures can improve virus adsorption by polyvalent cation enhancement or the use of alternative adsorbent filters, and can also enhance elution of adsorbed viruses.

Previous studies in this laboratory (described in the May 1980 JOURNAL[1]) revealed two major deficiencies of the Tentative Standard Method (TSM) for concentrating viruses from drinking water.[2] First, materials present in tap water interfered with virus recovery, either by clogging primary adsorbents prematurely or by inhibiting virus adsorption to filters. Virus adsorption interference was related to the volume of tap water processed, and could be overcome to some extent by removal of soluble or colloidal materials from tap water with granular activated carbon (GAC).

Second, representative viruses from the four major groups of enteric viruses were found to be rapidly inactivated when exposed to glycine-NaOH at pH 11.5, the eluent specified in the TSM. Thus the poor virus recoveries by the TSM that were observed[1] can be at least partially explained by virus inactivation during elution of adsorbent filters.

The purpose of the present study was to explore possible modifications of TSM methodology that would overcome these problems. Specifically, methods to improve virus adsorption by polyvalent cation enhancement[3-5] or use of alternative adsorbent filters and to improve elu-

0003-150X/80/060350-06$01.00
©1980 American Water Works Association

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

tion of adsorbed viruses were studied.

Materials and Methods

Viruses and cell cultures. The viruses employed in this study were laboratory strains of simian adenovirus SV-11 (SV-11), minute virus of mice (MVM, a parvovirus), reovirus type 3 (R-3), and poliovirus type 1, strain LSc (P-1). Viruses were produced, purified, and assayed as previously described.[1,7,8]

Virus concentration procedures. Single-step virus concentration experiments involving tap water volumes of 12 L or less were generally performed as previously described using 47-mm diameter virus adsorbent filter discs.[1] In some experiments small volumes of concentrated stock solutions of $MgCl_2$ or $AlCl_3$ were added to the tap water to desired final concentrations. The samples were filtered through either fiberglass-asbestos-epoxy* or fiberglass-epoxy† microporous filters (filters 1 and 2, respectively) at the desired flow rate using a regulated nitrogen gas cylinder as a source of positive pressure. When the virus inputs were high, the filtrates were sampled and assayed for viruses. Adsorbed viruses were eluted with 15-mL volumes of selected eluents. Beef extract eluents were prepared from beef extract powder.‡ Beef extract concentrations are expressed as weight percent (w/v); equivalent concentrations of beef extract solutions made from pastes would be approximately 20 percent higher. Elution was accomplished either by passing two successive 7.5-mL volumes of eluent through filters or by recirculating 15 mL of eluent through filters for 5 min by means of a peristaltic pump.

In virus concentration experiments involving 380 L (100 gal) or more, monodispersed viruses, sodium thiosulfate, HCl or NaOH, and any desired polyvalent cation salts were added to flowing tap water via a triplex fluid proportioner§ as previously described.[1] Conditioned seeded tap water was passed through 0.45-μm pore size filter 2 cartridge filters** [25 cm (10 in.) long by 7.5 cm (3 in.) in diameter] at 0.16 L/s (2.5 gpm). The filters were eluted with 1000-mL volumes of selected eluents, either by passing them once through the filter or by recirculating them through the filter for 5 min using a peristaltic pump. Primary eluates were reconcentrated by secondary adsorption-elution with 47-mm diameter, 0.45-μm porosity filter 2 disc filters[1,2] or organic flocculation.[9]

Results and Discussion

Enhancement of adsorption with polyvalent cations. The results of previous experiments[1] demonstrated that GAC was capable of removing from tap water soluble or colloidal components that interfere with virus adsorption to filters. However, GAC treatment of tap water prior to virus adsorption cannot be used as part of a concentration procedure for naturally occurring viruses in water because of the likelihood that the viruses would be removed by the GAC and thus never reach the adsorbent filter. Therefore, alternative methods to control these interfering components were investigated.

It has been previously shown that virus adsorption to filters in the presence of soluble or colloidal interferences can be enhanced by adding polyvalent cation salts to the water.[1,8] A series of experiments was conducted to determine whether the components in finished tap water interfering with primary virus adsorption in these studies could be overcome by this method. Volumes of dechlorinated seeded tap water [3×10^2 to 3×10^3 plaque-forming units (PFU) of virus per millilitre] with and without 5×10^{-4} M $AlCl_3$ were filtered at pH 3.5 through 47-mm diameter, 5 and 1-μm porosity filter 1 filter series. The filtrates were sampled to determine the percentage of initial viruses adsorbed to the filters. The results in Table 1 show that $AlCl_3$ at 5×10^{-4} M enhanced adsorption of SV-11, MVM, and R-3, with adsorption efficiencies being increased to >99 percent.

However, it should be noted that for SV-11 the presence of $AlCl_3$ often produced an apparent reduction in the number of infectious viruses initially present in pH 3.5 tap water, as compared to the number present in the control tap water containing no $AlCl_3$. This apparent reduction in virus infectivity could not be overcome by tenfold dilution of the sample in 0.1 M phosphate-buffered saline (PBS). It is not clear whether this observed decrease in SV-11 infectivity was due to aggregation, inactivation, or some other phenomenon. The factors responsible for this apparent virus reduction deserve further study.

In another experiment, replicate 6000-mL volumes of dechlorinated tap water with and without 5×10^{-4} M $MgCl_2$ were seeded with about 3×10^3 PFU of SV-11 per millilitre and filtered through 47-mm diameter, 5 and 1-μm porosity filter 1 filter series at pH 3.5, pH 5.0, and pH 6.0. Filtrate samples were taken for determination of the percentage of initial viruses adsorbed to the filters. As indicated by the results in Table 2, SV-11 adsorption was enhanced by $MgCl_2$. The extent of enhancement was somewhat pH-dependent, with decreased adsorption efficiency at the highest pH level tested. Thus, as in previous studies, the interfering effects of soluble or colloidal components on virus adsorption from water can be readily and expediently overcome by adding polyvalent cation salts.

Recovery of alkali-sensitive viruses with alternative eluents. The instability of the representative enteric viruses in pH 11.5 glycine-NaOH certainly contributed to the poor virus recoveries observed during this evaluation of the TSM.[1] In the case of the adenoviruses and reovirus, alkali sensitivity explains the apparent inability of the TSM to recover these viruses.[1] Experiments were conducted to find a more suitable eluting medium.

In the initial experiments, volumes of dechlorinated tap water were adjusted to pH 3.5 and seeded with known concentrations of SV-11, R-3, or MVM. The tap

TABLE 1
*Effect of $AlCl_3$ on Virus Adsorption to Filter 1 Filters**

Virus	Adsorption at pH 3.5, percent	
	No $AlCl_3$	5×10^{-4} M $AlCl_3$
SV-11	32	>99
R-3	97	>99
MVM	91	>99

*Filters—47-mm diameter, 5 and 1-μm porosity; volumes filtered—3000 mL for SV-11, 2000 mL for R-3, and 500 mL for MVM; tap water virus concentration—3×10^2 to 3×10^3 PFU/mL.

TABLE 2
*Effect of $MgCl_2$ on SV-11 Adsorption to Filter 1 Filters**

Adsorption pH	Adsorption, percent	
	No $MgCl_2$	5×10^{-4} M $MgCl_2$
3.5	31	99
5.0	73	99
6.0	50	81

*Filters—47-mm diameter, 5 and 1-μm porosity; volume filtered—6000 mL; tap water virus concentration—about 3×10^3 PFU/mL.

TABLE 3
*Effect of pH on Elution of Enteric Viruses From Filter 1 Filters With Glycine-NaOH**

Glycine-NaOH pH	Elution Efficiency, percent		
	MVM	R-3	SV-11
11.0–11.5	37	<1	<1
10.0–10.5	78	14	22
9.0–9.5	59	44	†
8.0–8.5	29	†	†
7.0–7.5	98	†	†

*Filters—47-mm diameter, 5 and 1-μm porosity; volumes filtered—500 mL for MVM, 2000 mL for R-3, and 500 mL for SV-11; tap water virus concentration—3×10^2 to 1×10^3 PFU/mL.
†Not tested

*Series AA, type M-780, Cox Instrument Div., Lynch Corp., Detroit, Mich.
†Filter 8025-035, Filterite Corp., Timonium, Md.
‡Lab Lemco Powder, Oxoid, K.C. Biologicals, Lexana Kan.
§Johanson and Son Machine Corp., Clifton, N.J.
**Duofine, Filterite Corp., Timonium, Md.

water volumes were then filtered through 47-mm diameter, 5 and 1-μm porosity filter 1 filter series, and filtrate samples were assayed for virus adsorption efficiency. Filter series were then eluted with 15-mL volumes of various eluent media. Elution efficiencies are expressed as the percentage of adsorbed viruses recovered in the eluate.

Glycine–NaOH. Glycine–NaOH at pH levels lower than 11.5 was evaluated as an alternative eluent. Enteric viruses at initial concentrations of about 3×10^2 to 1×10^4 PFU/mL in pH 3.5 tap water were adsorbed to replicate 47-mm diameter, 5 and 1-μm porosity filter 1 filters. Each filter was then eluted with 0.05 M glycine–NaOH at one of the pH levels shown in Table 3. Elution with pH 11.0–11.5 glycine–NaOH served as a control. For all viruses, elution efficiency was markedly improved at pH levels below 11.5. MVM elution was apparently optimal at pH 10.5, yielding 78 percent recovery of adsorbed viruses. However, an optimal pH for R-3 or SV-11 elution was not found because relatively few pH levels were tested. It is interesting to note that differences in elution efficiency between MVM, R-3, and SV-11 by pH 10–11.5 glycine–NaOH are reasonably consistent with the previously observed differences in the alkali sensitivity of these three viruses.[3] The results of these experiments verify that pH has a profound influence on the effectiveness of glycine–NaOH as a virus eluent, and that pH 11.5 is clearly not optimal for the viruses tested.

Although glycine–NaOH at pH levels below 11.5 yields improved elution efficiency, virus adsorption enhancement with polyvalent cations could interfere with virus desorption by the eluent, and thereby result in poor virus recovery. To investigate this possibility, MVM, R-3, and SV-11, at concentrations of 3×10^2 to 3×10^3 PFU/mL in pH 3.5 tap water with and without 5×10^{-4} M $AlCl_3$ or 5×10^{-3} M $MgCl_2$ were adsorbed to replicate 47-mm diameter, 5 and 1-μm porosity filter 1 filter series. The filters were then eluted with glycine–NaOH at pH 9.5–10.5. The results, as shown in Table 4, indicated that adsorption enhancement with $AlCl_3$ did not noticeably hinder subsequent elution of MVM or R-3. SV-11 elution was apparently unaffected by adsorption enhancement with $MgCl_2$; adsorption enhancement with $AlCl_3$ reduced subsequent virus elution efficiency by a factor of ten. Thus for at least some viruses reduction of elution efficiency may be a serious disadvantage of cation adsorption enhancement, especially with $AlCl_3$. This possibility deserves further investigation.

Beef extract. The use of proteinaceous media, such as beef extract, for virus elution has been suggested as an alternative to glycine–NaOH.[9,10] Studies were conducted to compare the elution efficiency of 2.4 percent beef extract and 0.05 M glycine–NaOH. MVM and R-3 at concentrations of 3×10^2 to 3×10^3 PFU/mL in pH 3.5 tap water were adsorbed to 47-mm diameter, 5 and 1-μm porosity filter 1 filter series. The adsorbed viruses were eluted from replicate filter series with equal volumes of one of these two eluents at pH levels of 9–9.5 and 10–10.5. As shown in Table 5, beef extract proved to be superior to glycine–NaOH at both pH levels tested. The reduced R-3 recovery with beef extract at pH 10–10.5, as compared to pH 9–9.5, may be caused by virus instability at the higher pH level.[4] In a similar experiment with SV-11, elution efficiency was 36 percent with 2.4 percent beef extract at pH 9, and 23 percent with glycine–NaOH at pH 10.

A problem associated with the use of proteinaceous media such as 2.4 percent beef extract for virus elution is eluent foaming that prevents complete recovery of eluate. Because less concentrated solutions of beef extract are less susceptible to foaming problems, an experiment was done to determine whether a reduction in the beef extract concentration would affect its virus elution effi-

TABLE 4
*Effect of Added Salts During Adsorption on Virus Elution From Filter 1 Filters With Glycine–NaOH**

Virus	Eluent pH	Elution Efficiency After Adsorption, percent		
		No Salts	5×10^{-4} M $AlCl_3$	5×10^{-3} M $MgCl_2$
MVM	10.5	78	68	†
SV-11	10.0	23	2	18
R-3	9.5	44	45	†

*Filters–47-mm diameter, 5 and 1-μm porosity; volumes filtered–500 mL for MVM, 1000 mL for SV-11, and 2000 mL for R-3; tap water pH–3.5; tap water virus concentration–3×10^2 to 3×10^3 PFU/mL.
†Not tested

TABLE 5
*Comparative Efficiency of 0.05 M Glycine–NaOH and 2.4 Percent Beef Extract as Eluents for MVM and R-3**

Virus	Elution Efficiency–percent			
	pH 9–9.5		pH 10–10.5	
	Glycine–NaOH	2.4 Percent Beef Extract	Glycine–NaOH	2.4 Percent Beef Extract
MVM	†	†	78	99
R-3	44	98	14	98

*Filters–47-mm diameter, 5 and 1-μm filter 1; volumes filtered–500 mL for MVM and 2000 mL for R-3; tap water virus concentration–3×10^2 to 3×10^3 PFU/mL; tap water pH–3.5.
†Not tested

TABLE 6
*Effect of pH 9.5 Beef Extract Concentration on SV-11 Elution From Filter 1 Filters**

Beef Extract Concentration, percent	Elution Efficiency, percent
0.2	42
0.5	46
1.0	59
2.4	26

*Tap water volume–500 mL; filters–47-mm diameter, 5 and 1-μm porosity; adsorption conditions–pH 3.5 with 0.0005 M $AlCl_3$; tap water virus concentration–about 3×10^2 PFU/mL; eluents were recirculated through the filters for 5 min.

TABLE 7
*Efficiency of Organic Flocculation for Enteric Viruses**

Virus	Initial Virus Recovered in Redissolved Sediment After Flocculation, percent		
	pH 3.5	pH 4.0	pH 4.5
SV-11	68	64	57
R-3	42	†	52
MVM	22	20	14

*Beef extract concentration–2.4 percent; initial virus concentration–10^2 to 10^3 PFU/mL; flocculation time–30 min.
†Not tested

TABLE 8
*Effect of Beef Extract Concentration on SV-11 Recovery by Organic Flocculation**

Beef Extract Concentration (w/v), percent	Initial Virus Detected, percent	
	Supernatant	Sediment
2.4	15	68
2.0	14	76
1.5	11	71
1.0	11	71
0.75	01	77
0.5	13	61
0.3	07	45
0.1	35	54

*Initial virus concentration–3×10^3 PFU/mL; flocculation pH–3.5; flocculation time–30 min.

TABLE 9
*Organic Flocculation of SV-11 in Primary Eluates**

Primary Filter	Beef Extract Concentration (w/v), percent	Initial Virus Recovered, percent	
		Supernatant	Sediment
1†	1.0	1.2	56
	1.5	1.5	57
	2.0	1.7	54
1‡	1.0	1.9	74
	2.0	2.7	90
4§	1.0	1.5	60
	2.0	1.5	56
5**	1.0	0.1	86
	2.0	0.1	99

*Tap water volume–380 L (100 gal); primary adsorption pH–3.5; eluent–0.2 percent beef extract-0.05 M glycine-NaOH; virus concentration in primary eluate–5×10^3 PFU/mL; organic flocculation pH–3.5; 30 min.
†Cox Instrument Div., Lynch Corp., Detroit, Mich.
‡Millipore Corp., Bedford, Mass.
§Carborundum Co., Lebanon, Ind., and Cox Instrument Div., Lynch Corp., Detroit, Mich.
**Balston, Inc., Lexington, Mass.

the two negatively charged surfaces.[12,13]

Microporous filters that are more positively charged in the pH range of most natural and tap waters (pH 5–9) would appear to offer advantages over negatively charged filters as virus adsorbents by reducing or eliminating the need for either acid or salt addition to obtain virus adsorption. The different chemical characteristics of these more electropositive filters, compared to the conventional electronegative filters, might also make the former less susceptible to virus adsorption interference by soluble or colloidal constituents of tap water and other waters. Furthermore, if the filters became negatively charged at pH levels somewhat higher than those of tap waters, it might be possible to elute the adsorbed viruses under moderately alkaline conditions. Therefore, two general types of more electropositive filter media were studied as alternative adsorbents: cellulose-diatomaceous earth ("charged modified") resin* and asbestos-cellulose resin.† The results of these investigations, which have been reported elsewhere,[15,16] showed that positively charged microporous filters, especially surface-modified cellulose-diatomaceous earth, can yield efficient virus recovery when used in adsorption–elution concentration methodology.

Summary and Conclusions

Modifications of the TSM greatly improved enteric virus recovery efficiency. The interference with virus adsorption to the filters by tap water constituents was overcome by adding polyvalent cations, specifically $MgCl_2$ or $AlCl_3$, to the tap water before filtration. Potential problems associated with polyvalent cation adsorption enhancement include the pH dependency of enhancement and interference by $AlCl_3$ with elution of some viruses from the adsorbents; these areas deserve further investigation.

*Zeta Plus, AMF Corp., Cuno Div., Meriden, Conn.
†Seitz, Alsop Engineering Corp., Milldale, Conn.

TABLE 14
*Concentration of SV-11 From 380 L (100 gal) of Tap Water**

Run Number	Virus Input PFU	Virus Recovery percent
1	219	46
2	129	89
3	123	109
4	40	38
5	431	61
6	40	20
7	40	18
8	171	44
9	229	54
Mean	158	53

*Primary concentration–adsorption to filter 2 filters at pH 3.5 with 5×10^{-3} M $MgCl_2$, elution with 0.2 percent beef extract in 0.05 M glycine at pH 9.5, recirculated for 5 min; secondary concentration–organic flocculation with 1.5 percent beef extract at pH 3.5.

The use of alternative adsorbent filters can circumvent some deficiencies in the TSM. Filter 2 0.45-μm fiberglass-epoxy filters are capable of processing larger volumes of tap water without clogging than are some of the TSM-recommended adsorbents. Moreover, virus recovery efficiency by filter 2 filters, especially when used with certain procedural modifications, is comparable or superior to recovery efficiencies by TSM-recommended adsorbents. Similarly, filter media that are more positively charged than TSM adsorbents in the pH range of tap water appear promising as alternative virus adsorbents.[15]

Elution of alkali-sensitive enteric viruses can be significantly improved by the use of alternative eluting media. Glycine-NaOH at pH levels less alkaline than those recommended in the TSM (pH 11.5) showed superior elution efficiency for all viruses examined. Beef extract at pH 9.5–10.5 and concentrations of 0.2–2.4 percent efficiently eluted enteric viruses. Reconcentration of beef extract eluates by organic flocculation was relatively efficient for SV-11 and R-3. The efficiency of the organic flocculation method was found to be relatively independent of beef extract concentration or flocculation time. Some possible problems with organic flocculation reconcentration that warrant further study include the pH dependency of flocculation efficiency and the relatively low recovery efficiency for some viruses such as MVM.

The results of this study show that modifications of the TSM are necessary to improve the efficiency of filter adsorption–elution methods for virus concentration from tap water. The procedural modifications described here are improvements, but they are by no means final. They do, however, indicate directions in which future investigations should proceed. It should be noted that many of these procedural modifications for improved virus recoveries from large volumes of finished water have been incorporated in the 15th edition of *Standard Methods for the Examination of Water and Wastewater*.[17]

Acknowledgments

We thank M.F. Kennell for technical assistance and Stephen E. Oglesbee for the preparation of cell cultures. This work was supported by a grant (R804218) from the US Environmental Protection Agency. M.D. Sobsey is a recipient of a research career development award (5K04ES00026) from the National Institute of Environmental Health Sciences.

References

1. Sobsey, M.D. et al. Evaluation of the Tentative Standard Method for Enteric Virus Concentration From Large Volumes of Tap Water. *Jour. AWWA*, 72:5:292 (May 1980).
2. *Standard Methods for the Examination of Water and Wastewater*. APHA, AWWA, and WPCF, Washington, D.C. (14th ed., 1976).
3. Sobsey, M.D. Detection Methods for Enteric Viruses in Water and Wastewater. *Viruses in Water* (G. Berg, et al, editors). APHA, Washington, D.C. (1976).
4. Homma, A. et al. Virus Concentration from Sewage. *Water Res.*, 7:7:945 (Sep. 1973.)
5. Sobsey, M.D. et al. Concentration of Enteroviruses From Large Volumes of Water. *Appl. Microbiol.*, 26:4:529 (Oct. 1973).
6. Wallis, C. & Melnick, J. L. Concentration of Viruses from Sewage by Adsorption on Millipore Membranes. *Bull. World Health Org.*, 36:2:219 (Aug. 1967).
7. Sobsey, M.D.; Jensen, H.R.; & Carrick, R.J. Improved Methods for Detecting Enteric Viruses in Oysters. *Appl. Envir., Microbiol.*, 36:1:121 (Jul. 1978).
8. Floyd, R., & Sharp, D.G. Aggregation of Poliovirus and Reovirus by Dilution in Water. *Appl. Envir. Microbiol.*, 33:1:159 (Jan. 1977).
9. Katzenelson, E.; Fattal, B.; & Hostovesky, T. Organic Flocculation: An Efficient Second-Step Concentration Method for the Detection of Viruses in Tapwater. *Appl. Envir. Microbiol.*, 32:4:638 (Oct. 1976).
10. Fields, H.A. & Metcalf, T.G. Concentration of Adenovirus from Seawater. *Water Res.*, 9:357 (Apr. 1975).
11. Farrah, S.R. et al. Concentration of Viruses from Large Volumes of Tapwater Using Pleated Membrane Filters. *Appl. Envir. Microbiol.*, 31:2:221 (Feb. 1976).
12. Valentine, R.C. & Allison, A.C. Viral Particle Adsorption. I. Theory of Adsorption and Experiments on the Attachment of Particles to Non-Biological Surfaces. *Biochim. Biophys. Acta*, 34:10 (1959).
13. Kessick, M.A. & Wagner, R.A. Electrophoretic Mobilities of Virus Adsorbing Filter Media. *Water Res.*, 12:263 (Apr. 1978).
14. Mandel, B. Characterization of Type 1 Poliovirus by Electrophoretic Analysis. *Virol.*, 44:554 (May 1971).
15. Sobsey, M.D. & Jones, B.L. Concentration of Poliovirus from Tap Water Using Positively Charged Microporous Filters. *Appl. Envir. Microbiol.*, 37:3:588 (Mar. 1979).
16. Glass, J.S. & Sobsey, M.D. Poliovirus Concentration From Large Volumes of Water With Electropositive Cartridge Filters. Abstract Q135, Abstracts of the 1980 Annual Meeting of the American Soc. for Microbiology. American Soc. for Microbiology, Washington, D.C. (1980).
17. *Standard Methods for the Examination of Water and Wastewater*. APHA, AWWA, and WPCF, Washington, D.C. (15th ed., in press).

A paper contributed to and selected by the Journal, authored by Mark D. Sobsey, assoc. prof., J. Steven Glass, res. asst., and Robert R. Jacobs and William A. Rutala, doctoral candidates, all of the dept. of envir. sci. and engrg., school of public health, Univ. of North Carolina, Chapel Hill, N.C.

11190 4230, 4311

Reprinted and copyrighted as a part of
JOURNAL AMERICAN WATER WORKS ASSN
Vol. 72 No. 6 June 1980
Printed in U.S.A.

Preliminary experiments were conducted to evaluate 0.45-μm porosity filter 2 filters as adsorbents for two viruses; specifically, P-1 and SV-11. In the initial experiments 3000-mL volumes of dechlorinated, pH 3.5 tap water, with and without 5×10^{-3} M $MgCl_2$ or 5×10^{-4} M $AlCl_3$, were seeded with about 3×10^5 PFU of viruses per millilitre and filtered through 47-mm diameter filter 2 discs. Filtrates were sampled for determination of virus adsorption efficiency. The filters were then eluted with 15-mL volumes of 0.05 M glycine and 0.005–0.01 M ethylenediamine tetraacetic acid (EDTA) at pH 9.0–10.0. EDTA was included in the eluent with the idea of complexing polyvalent cations possibly involved in mediating virus adsorption,[12,13] and thereby improving elution. As shown in Table 11, adsorption of both viruses to fiberglass filters was markedly enhanced by added polyvalent cations. Moreover, adsorption of SV-11 to filter 2 filters at pH 3.5 was comparable to, if not greater than, adsorption efficiencies observed with 5 and 1-μm porosity filter 1 filter series (Tables 1 and 2). Elution of viruses was efficient but varied according to the presence and type of salt during adsorption. Interestingly, elution of P-1 was more efficient after cation-enhanced adsorption. Elution of SV-11 from filter 2 (Table 11) appeared more efficient than elution from filter 1 filters (Table 4).

In order to further compare virus elution efficiencies from filters 1 and 2, to evaluate the influence of EDTA on elution, and to investigate the effects of prolonged eluent contact with filters, SV-11 at a concentration of about 3×10^5 PFU per millilitre of tap water was adsorbed with 5×10^{-3} M $MgCl_2$ or 5×10^{-4} M $AlCl_3$ to parallel 47-mm diameter filter 1 and 2 discs. The filters were eluted at pH 10 with 0.05 M glycine–NaOH or with glycine–NaOH containing 0.005 M EDTA. In addition, filter 2 discs were eluted either by successive passes of two 7.5-mL volumes of eluent or by recirculating, by means of a peristaltic pump, 15 mL of eluent through the filter for 5 min. The results (Table 12) verified that SV-11 is eluted more efficiently from filter 2 than from filter 1 filters at pH 10, and that elution efficiency is dependent upon the polyvalent cation used for adsorption enhancement, with more efficient elution of viruses adsorbed with $MgCl_2$ than with $AlCl_3$. Neither the presence of 0.005 M EDTA in the glycine eluent nor prolonged eluent contact by recirculation significantly improved SV-11 elution from filter 2 filters. The results of these small-volume experiments suggested that filter 2 fiberglass filters may be substituted for TSM-recommended filter 1 filters with no decrease, and a possible increase, in virus concentration efficiency. Large-volume experiments were conducted to verify this finding.

In one series of experiments 10^7 PFU of MVM and 10^8 PFU of P-1 were simultaneously added to 1000-L volumes of dechlorinated tap water via one pump of the triplex fluid proportioner, as previously described for large-volume TSM evaluations.[1] The remaining two pumps added HCl to bring the tap water to pH 3.5. In some experiments $MgCl_2$ or $AlCl_3$ was added along with acid to final concentrations in tap water of 5×10^{-3} M or 5×10^{-4} M, respectively. After virus addition and pH adjustment, the seeded conditioned tap water was passed through 0.45-μm porosity filter 2 cartridges. Filtrate samples were collected for determination of unadsorbed viruses after volumes of 10 L, 100 L, 300 L, and 1000 L had been processed through the adsorbent. After processing 1000 L of tap water, filters were eluted by one of two methods. For filters receiving tap water with no added salts or $MgCl_2$, elution was done by passing 1000 mL of glycine–NaOH at pH 11.0 through the cartridge; the eluates were immediately neutralized by collection in 500 mL of pH 2.0 glycine–HCl. Filters receiving tap water with $AlCl_3$ were eluted by recirculating glycine–NaOH with 0.2 percent beef extract at pH 9.5 through the cartridges for 5 min. The results of these experiments are summarized in Table 13. Adsorption of P-1 and MVM to filter 2 cartridges followed a pattern similar to that observed in the small-volume studies, in that adsorption was improved for both viruses by the addition of $MgCl_2$ and $AlCl_3$. Similarly, elution of these two viruses from filter 2 cartridges was more efficient after adsorption in the presence of polyvalent cations, as was the case in small-volume experiments (Table 11). Notably, filter 2 cartridges showed no tendency to clog, even after processing 1000 L of tap water.

Large-volume experiments were also done using filter 2 cartridges and low levels of SV-11. Tap water in 380-L (100-gal) volumes, with or without 5×10^{-3} M added $MgCl_2$ and seeded with 40–431 PFU of SV-11, were filtered at pH 3.5 through filter 2 cartridges. The filters were eluted by recirculating 0.2 percent beef extract–0.05 M glycine at pH 9.5 through the cartridges for 5 min. Eluates were adjusted to 1.5 percent beef extract by adding an appropriate amount of a 10 percent stock solution, and then reconcentrated by organic flocculation at pH 3.5. When adsorption was enhanced with 5×10^{-3} M $MgCl_2$, the SV-11 recovery ranged from 18 to 109 percent, and averaged 53 percent (Table 14). In similar experiments without $MgCl_2$ adsorption enhancement, SV-11 recovery was only 18 percent. Thus use of 0.45-μm porosity filter 2 cartridges with procedural modifications of primary adsorption enhancement with $MgCl_2$, elution by recirculation of glycine–beef extract, and reconcentration by organic flocculation yielded relatively efficient virus recovery compared to the TSM,[1] without the disadvantages of filter clogging.

Electropositive filters. Recent electrophoretic studies have established that the microporous filters now employed for concentrating viruses from water are negatively charged throughout the pH range of 2 to 7, with the net negative charge approaching neutrality below pH 2.[13] Electrophoretic studies of viruses indicate that most viruses are also negatively charged near neutral pH and have isoelectric points below pH 7.[14] The fact that both adsorbent filters and viruses are negatively charged near neutral pH may explain the need for acidification or the addition of polyvalent cations to obtain efficient virus adsorption from water. Adsorption may be more efficient at pH 3.5 than at pH 7.5 because there is likely to be electrostatic attraction between viruses and filters at the lower pH, whereas there is electrostatic repulsion near neutral pH. Near neutral pH, polyvalent cations could serve to mediate the adsorption reaction between

TABLE 13

Concentration of Poliovirus and MVM From 1000 L of Tap Water Using Filter 2 Cartridges

Adsorption Conditions		Viruses Adsorbed* percent			Viruses Eluted† percent	
Salt	Concentration M	P-1	MVM	Eluent	P-1	MVM
None		45	49	glycine-NaOH at pH 11.0‡	8.2	2.0
$MgCl_2$	5×10^{-3}	60	73	glycine-NaOH at pH 11.0‡	46	38
$AlCl_3$	5×10^{-4}	99	97	glycine-beef extract at pH 9.5§	22	22

*Adsorption at pH 3.5; percent adsorbed calculated by subtracting volume-weighted sum of viruses in filtrate samples from total input viruses (10^7 PFU of MVM and 10^8 PFU of P-1)
†Percent of adsorbed viruses eluted
‡Single pass, 1000 mL glycine-NaOH, pH 11.0
§Glycine-0.2 percent beef extract recirculated 5 min

ciency. SV-11 at a concentration of 3×10^5 PFU/mL in pH 3.5 tap water containing 5×10^{-4} M $AlCl_3$ was adsorbed to replicate 47-mm diameter, 5 and 1-μm porosity filter 1 filter series. The adsorbed viruses were eluted with equal volumes of pH 9.5 beef extract ranging in concentration from 0.2 to 2.4 percent. The results (Table 6) showed no decrease in elution efficiency as the beef extract concentration was reduced from 2.4 to 0.2 percent. Moreover, at concentrations less than 1 percent, beef extract foaming is minimal. The results of these experiments suggested that beef extract at low concentrations and moderately alkaline pH levels may be an excellent alternative eluent, and further experiments were performed in an effort to optimize conditions for its use.

Beef extract eluates cannot be efficiently reconcentrated by secondary filter adsorption–elution because the protein content interferes with virus adsorption. An alternative reconcentration technique for beef extract eluates is organic flocculation,[9] and the efficiency of this method was investigated. Viruses were added to replicate 20 or 200-mL volumes of 2.4 percent beef extract to give an initial concentration between 10^3 and 10^4 PFU/mL, and samples were taken to determine initial virus titers. The contaminated beef extract samples were then acidified with HCl to the pH levels shown in Table 7 and stirred slowly for 30 min. The resulting floc was removed by slow-speed centrifugation, redissolved in about 5 mL of 0.15 M Na_2HPO_4 buffer, adjusted to pH 7.4 with additional buffer (if necessary), and then assayed to determine virus recovery. The results (Table 7) indicate that organic flocculation at pH 3.5–4.5 can concentrate SV-11 and R-3 with recovery efficiencies of about 50 to 60 percent. In the case of MVM, however, the technique was less effective than for the other two viruses at all flocculation pH levels tested, with recovery efficiencies lower than 25 percent. Thus modifications of the organic flocculation method may be necessary to improve recovery efficiency for some enteric viruses.

Further investigations to optimize the organic flocculation technique were conducted using SV-11. To determine the influence of beef extract concentration on organic flocculation efficiency, SV-11 was added to a concentration of about 3×10^5 PFU/mL to 200-mL volumes of beef extract ranging in concentration from 0.1 to 2.4 percent. The samples were acidified with HCl to pH 3.5 and stirred slowly for 30 min. After floc removal by slow-speed centrifugation, supernatants were sampled to determine the fraction of initial viruses not adsorbed to the floc. The floc sediments were redissolved in 7.5 mL of 0.15 M Na_2HPO_4, giving a final pH of about 7.5, and then assayed to determine SV-11 recovery efficiency. As shown in Table 8, virus adsorption and recovery efficiencies were similar for beef extract concentrations between 2.4 and 0.75 percent. At concentrations lower than 0.75 percent only marginal decreases in adsorption and recovery were observed, despite the fact that no visible floc was formed upon acidification of the least concentrated beef extract solution.

A similar experiment was performed with glycine eluates obtained from TSM primary adsorbents that had processed 380 L (100 gal) of tap water. The eluates were divided into 200-mL aliquots, and beef extract was added in the form of a 10 percent concentrate solution to the final concentrations shown in Table 9. Each aliquot was seeded with about 5×10^2 PFU of SV-11 per millilitre and subjected to organic flocculation. As in the previous experiments, the beef extract concentration had no discernible influence on the efficiency of reconcentration by organic flocculation (Table 9). Moreover, the SV-11 recoveries by organic flocculation were comparable to the recoveries obtained in the previous experiments with beef extract in distilled water, averaging 73 percent. Thus the presence of glycine did not adversely affect the efficiency of SV-11 recovery by organic flocculation.

The effect of flocculation time on SV-11 recovery by organic flocculation was also investigated. Replicate 200-mL volumes of 1.5 percent beef extract in 0.05 M glycine were contaminated with about 3×10^5 PFU of SV-11 per millilitre. Samples were then adjusted to pH 3.5 and mixed for time periods of <1, 5, 15, and 30 min before removal of the floc by slow-speed centrifugation. As in previous experiments, both the supernatant and redissolved floc sediment were sampled. The results (Table 10) showed that the flocculation time had no noticeable influence on SV-11 removal from the supernatant or recovery in the sediment. The recoveries ranged from 43 to 50 percent, and averaged 47 percent.

The results of the foregoing experiments indicate that the organic flocculation reconcentration method is quite effective and flexible, and with some further improvements for certain viruses such as MVM, it may prove to be an excellent alternative to secondary filter adsorption–elution for reconcentration of viruses in primary adsorbent eluates.

Alternative primary adsorbent filters. Each of the primary adsorbent filters recommended in the TSM has inherent disadvantages.* Thus two alternative primary adsorbent filters were investigated.

Fiberglass cartridges. Fiberglass filters† are available in 0.45-μm porosity as sheet material and as 25-cm (10-in.) long by 7.5-cm (3-in.) diameter pleated sheet cartridges‡ containing about 0.5 m^2 (5 sq ft) of surface area. Cartridges have been used previously as virus adsorbents,[11] and studies in the authors' laboratory showed that cartridges are capable of processing at least 1000 L of Chapel Hill tap water without clogging.

TABLE 10
*Effect of Flocculation Time on SV-11 Concentration by Organic Flocculation**

Flocculation Time–min	Initial Virus Detected percent	
	Supernatant	Sediment
< 1	< 0.4	50
5	0.5	49
15	0.2	43
30	< 0.4	47

*Beef extract–1.5 percent in 0.05 M glycine; initial virus concentration–3×10^5 PFU/mL.

TABLE 11
*Concentration of Poliovirus and SV-11 With 0.45-μm Filter 2 Filters**

Virus	Filtration Mode	Adsorption Efficiency at pH 3.5		
		No Salts	5×10^{-4} M $MgCl_2$	5×10^{-4} M $AlCl_3$
P-1	adsorption†	40	99	99
	elution‡	45	99	83
SV-11	adsorption	45	99	99
	elution	§	69	32

*Tap water volume filtered–3785 mL for P-1, 3000 mL for SV-11; tap water virus concentration–about 3×10^5 PFU/mL; tap water pH–3.5
†Initial viruses adsorbed (percent)
‡Adsorbed viruses eluted (percent) with 0.05 M glycine at pH 9.5–10.0 with EDTA (0.005 M for SV-11, 0.01 M for P-1)
§ Not tested

TABLE 12
*Elution of SV-11 From Filters 1 and 2 With Glycine-NaOH and Glycine-NaOH-EDTA at pH 10**

Adsorption Conditions				Elution Efficiency percent	
Salt	Concentration M	EDTA†	Repeated Elutions	Filter 1	Filter 2
$AlCl_3$	5×10^{-4}	–	–	24	14
$AlCl_3$	5×10^{-4}	+	–	30	22
$MgCl_2$	5×10^{-4}	–	–	18	64
$MgCl_2$	5×10^{-4}	+	–	22	69
$AlCl_3$	5×10^{-4}	–	+	‡	40
$AlCl_3$	5×10^{-4}	+	+	‡	22
$MgCl_2$	5×10^{-4}	–	+	‡	52
$MgCl_2$	5×10^{-4}	+	+	‡	69

*Tap water volume–1.0 L; tap water pH–3.5; tap water virus concentration–3×10^5 PFU/mL
†EDTA concentration–0.005 M
‡Not done

*Balston tubes (Balston, Inc., Lexington, Mass.) generally yielded poorer virus recoveries than the other three filters; Cox M-780 AA-type filters (Cox Instrument Div., Lynch Corp., Detroit, Mich.) are no longer commercially available; and, perhaps most critical, Millipore membranes (Millipore Corp., Bedford, Mass.) and Carborundum K27 (Commercial Filters Div., Carborundum Co., Lebanon, Ind.)–Cox M-780 filter series were quite susceptible to clogging by materials in tap water.
†Filterite Corp., Timonium, Md.
‡Duofine, Filterite Corp., Timonium, Md.